

PATENT APPLICATION

**USES OF IL-23 AGONISTS AND ANTAGONISTS; RELATED REAGENTS**

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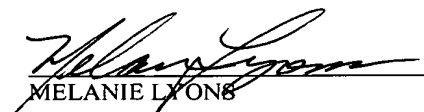
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"Express Mailing" mailing label number EL 982 851 094 US

Date of Deposit March 9, 2004

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## USES OF IL-23 AGONISTS AND ANTAGONISTS; RELATED REAGENTS

[0001] This application claims benefit from U.S. Provisional Patent Application No. 60/453,672, filed March 10, 2003.

## FIELD OF THE INVENTION

[0002] The present invention concerns uses of mammalian cytokine molecules and related reagents. More specifically, the invention relates to identification of mammalian cytokine-like proteins and inhibitors thereof that can be used in the treatment of proliferative disorders.

## BACKGROUND OF THE INVENTION

[0003] Cancers and tumors can be controlled or eradicated by the immune system. The immune system includes several types of lymphoid and myeloid cells, e.g., monocytes, macrophages, dendritic cells (DCs), eosinophils, T cells, B cells, and neutrophils. These lymphoid and myeloid cells produce secreted signaling proteins known as cytokines. The cytokines include, e.g., interleukin-10 (IL-10), interferon-gamma (IFN $\gamma$ ), IL-12, and IL-23. Immune response includes inflammation, i.e., the accumulation of immune cells systemically or in a particular location of the body. In response to an infective agent or foreign substance, immune cells secrete cytokines which, in turn, modulate immune cell proliferation, development, differentiation, or migration. Immune response can produce pathological consequences, e.g., when it involves excessive inflammation, as in the autoimmune disorders, whereas impaired immune response may result in cancer. Anti-tumor response by the immune system includes innate immunity, e.g., as mediated by macrophages, NK cells, and neutrophils, and adaptive immunity, e.g., as mediated by antigen presenting cells (APCs), T cells, and B cells (see, e.g., Abbas, *et al.* (eds.) (2000) *Cellular and Molecular Immunology*, W.B. Saunders Co., Philadelphia, PA; Oppenheim and Feldmann (eds.) (2001) *Cytokine Reference*, Academic Press, San Diego, CA; von Andrian and Mackay (2000) *New Engl. J. Med.* 343:1020-1034; Davidson and Diamond (2001) *New Engl. J. Med.* 345:340-350).

**[0004]** Methods of modulating immune response have been used in the treatment of cancers, e.g., melanoma. These methods include treatment with cytokines or anti-cytokine antibodies, such as IL-2, IL-12, tumor necrosis factor-alpha (TNFalpha), IFNgamma, granulocyte macrophage-colony stimulating factor (GM-CSF), and transforming growth factor (TGF). Where a cancer cell can produce a cytokine that enhance its own growth or its own survival, an anti-cytokine antibody may be an appropriate therapeutic agent (see, e.g., Ramirez-Montagut, *et al.* (2003) *Oncogene* 22:3180-3187; Braun, *et al.* (2000) *J. Immunol.* 164:4025-4031; Shaw, *et al.* (1998) *J. Immunol.* 161:2817-2824; Coussens and Werb (2002) *Nature* 420:860-867; Baxevas, *et al.* (2000) *J. Immunol.* 164:3902-3912; Shimizu, *et al.* (1999) *J. Immunol.* 163:5211-5218; Belardelli and Ferrantini (2002) *TRENDS Immunol.* 23:201-208; Seki, *et al.* (2002) *J. Immunol.* 168:3484-3492; Casares, *et al.* (2003) *J. Immunol.* 171:5931-5939; Oft, *et al.* (2002) *Nature Cell Biol.* 4:487-494)

**[0005]** Interleukin-23 (IL-23) is a heterodimeric cytokine comprised of two subunits, i.e., p19 and p40. The p19 subunit is structurally related to IL-6, granulocyte-colony stimulating factor (G-CSF), and the p35 subunit of IL-12. The p40 subunit of IL-23 is also part of IL-12, a heterodimeric cytokine comprising p35 and p40. IL-23 mediates signaling by binding to a heterodimeric receptor, comprised of IL-23R and IL-12beta1. The IL-12beta1 subunit is shared by the IL-12 receptor, which is composed of IL-12beta1 and IL-12beta2. A number of early studies demonstrated that the physiological consequences of a genetic deficiency in p40 (p40 knockout mouse; p40KO mouse; p40<sup>-/-</sup> mouse) were different from, e.g., more severe or less severe, than those found in a p35KO mouse. Some of these results were eventually explained by the discovery of IL-23, and the finding that the p40KO prevents expression of both IL-12 and IL-23 (Oppmann, *et al.* (2000) *Immunity* 13:715-725; Wiekowski, *et al.* (2001) *J. Immunol.* 166:7563-7570; Parham, *et al.* (2002) *J. Immunol.* 168, 5699-708; Frucht (2002) *Sci STKE* 2002, E1-E3; Elkins, *et al.* (2002) *Infection Immunity* 70:1936-1948; Cua, *et al.* (2003) *Nature* 421:744-748).

**[0006]** Present methods for treating cancer are not completely effective, and cytokines, such as IL-12 or IFNgamma produce toxic side effects (see, e.g., Naylor and Hadden (2003) *Int. Immunopharmacol.* 3:1205-1215; Fernandez, *et al.* (1999) *J. Immunol.* 162:609-617). The present invention addresses these problems by providing methods of using agonists and antagonists of IL-23.

## SUMMARY OF THE INVENTION

[0007] The present invention is based upon the discovery that an agonist or antagonist of IL-23 can modulate tumor growth.

[0008] The present invention provides a method of modulating tumor growth comprising contacting a tumor cell with an effective amount of an agonist or antagonist of IL-23. Also provided is the above method, wherein the antagonist of IL-23 inhibits or prevents tumor growth; as well as the above method wherein the tumor cell expresses IL-23. In another aspect, the present invention provides the above method wherein the agonist or antagonist of IL-23 comprises a binding composition that specifically binds a polypeptide or nucleic acid of p19 (SEQ ID NOs:1, 2, 3, or 4); or IL-23R (SEQ ID NOs:5 or 6); or the above method wherein the binding composition comprises: an antigen-binding site of an antibody; an extracellular region of IL-23R (SEQ ID NOs:5 or 6); a small molecule; an anti-sense nucleic acid or small interference RNA (siRNA); or a detectable label; and the above method wherein the binding composition comprises: a polyclonal antibody; a monoclonal antibody; a humanized antibody, or a fragment thereof; an Fab, Fv, or F(ab')<sub>2</sub> fragment; or a peptide mimetic of an antibody.

[0009] Yet another embodiment of the present invention provides a method of modulating tumor growth comprising contacting a tumor cell with an effective amount of an agonist or antagonist of IL-23; wherein the tumor cell is: a colon cancer cell; an ovarian cancer cell; a breast cancer cell; or a melanoma cell.

[0010] In another aspect, the invention provides a method of treating a subject suffering from a cancer or tumor comprising administering to the subject an effective amount of an agonist or antagonist of IL-23; and the above method wherein the antagonist of IL-23 inhibits: growth of the cancer or tumor; cachexia; anorexia; or angiogenesis. Also provided is the above method wherein the antagonist of IL-23 comprises a binding composition that specifically binds a polypeptide or nucleic acid of: p19 (SEQ ID NOs:1, 2, 3, or 4) or IL-23R (SEQ ID NOs:5 or 6). Yet another embodiment of the present invention provides the above method wherein the binding composition comprises: an antigen-binding site of an antibody; an extracellular region of IL-23R (SEQ ID NOs:5 or 6); an anti-sense nucleic acid or small interference RNA (siRNA); a small molecule; or a detectable label; and the above method wherein the binding composition comprises: a polyclonal antibody; a monoclonal antibody; a

humanized antibody, or a fragment thereof; an Fab, Fv, or F(ab')<sub>2</sub> fragment; or a peptide mimetic of an antibody.

**[0011]** In another embodiment, the invention provides the above method wherein the cancer or tumor is of the: gastrointestinal tract; respiratory tract; reproductive system; or endocrine system; as well as the above method wherein the cancer or tumor is: colon cancer; ovarian cancer; a melanoma; or breast cancer.

**[0012]** In other aspect of the present invention provides a method of diagnosis of a cancer or tumor comprising contacting a sample from a subject with the binding compositions of the above method, as well as the above method of diagnosis, wherein the binding composition comprises a nucleic acid probe or primer that specifically binds or hybridizes to the polynucleotide of SEQ ID NOs:1, 2, or 5.

**[0013]** Yet another embodiment of the present invention provides a kit for the diagnosis of a cancer or tumor comprising the binding composition of the above method and a compartment or instructions for use or disposal. Also provided is the above kit wherein the binding composition comprises an antibody that specifically binds to p19 (SEQ ID NOs:1, 2, 3, or 4) or IL-23R (SEQ ID NOs:5 or 6).

## DETAILED DESCRIPTION

**[0014]** As used herein, including the appended claims, the singular forms of words such as “a,” “an,” and “the,” include their corresponding plural references unless the context clearly dictates otherwise. All references cited herein are incorporated by reference to the same extent as if each individual publication, patent application, or patent, was specifically and individually indicated to be incorporated by reference.

I. Definitions.

**[0015]** “Activation,” “stimulation,” and “treatment,” as it applies to cells or to receptors, may have the same meaning, e.g., activation, stimulation, or treatment of a cell or receptor with a ligand, unless indicated otherwise by the context or explicitly. “Ligand” encompasses natural and synthetic ligands, e.g., cytokines, cytokine variants, analogues, muteins, and binding compositions derived from antibodies. “Ligand” also encompasses small molecules, e.g., peptide mimetics of cytokines and peptide mimetics of antibodies. “Activation” can refer to cell activation as regulated by internal mechanisms as well as by external or environmental factors. “Response,” e.g., of a cell, tissue, organ, or organism, encompasses a change in biochemical or physiological behavior, e.g., concentration, density, adhesion, or migration within a biological compartment, rate of gene expression, or state of differentiation, where the change is correlated with activation, stimulation, or treatment, or with internal mechanisms such as genetic programming.

**[0016]** “Activity” of a molecule may describe or refer to the binding of the molecule to a ligand or to a receptor, to catalytic activity; to the ability to stimulate gene expression or cell signaling, differentiation, or maturation; to antigenic activity, to the modulation of activities of other molecules, and the like. “Activity” of a molecule may also refer to activity in modulating or maintaining cell-to-cell interactions, e.g., adhesion, or activity in maintaining a structure of a cell, e.g., cell membranes or cytoskeleton. “Activity” can also mean specific activity, e.g., [catalytic activity]/[mg protein], or [immunological activity]/[mg protein], concentration in a biological compartment, or the like. “Proliferative activity” encompasses an activity that promotes, that is necessary for, or that is specifically associated with, e.g., normal cell division, as well as cancer, tumors, dysplasia, cell transformation, metastasis, and angiogenesis.

[0017] “Administration” and “treatment,” as it applies to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, compound, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. “Administration” and “treatment” can refer, e.g., to therapeutic, placebo, pharmacokinetic, diagnostic, research, and experimental methods. “Treatment of a cell” encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. “Administration” and “treatment” also means *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding composition, or by another cell. “Treatment,” as it applies to a human, veterinary, or research subject, refers to therapeutic treatment, prophylactic or preventative measures, to research and diagnostic applications. “Treatment” as it applies to a human, veterinary, or research subject, or cell, tissue, or organ, encompasses contact of an IL-23 agonist or IL-23 antagonist to a human or animal subject, a cell, tissue, physiological compartment, or physiological fluid. “Treatment of a cell” also encompasses situations where the IL-23 agonist or IL-23 antagonist contacts IL-23 receptor (heterodimer of IL-23R and IL-12Rbeta1), e.g., in the fluid phase or colloidal phase, as well as situations where the agonist or antagonist contacts a fluid, e.g., where the fluid is in contact with a cell or receptor, but where it has not been demonstrated that the agonist or antagonist contacts the cell or receptor.

[0018] “Binding composition” refers to a molecule, small molecule, macromolecule, antibody, a fragment or analogue thereof, or soluble receptor, capable of binding to a target. “Binding composition” also may refer to a complex of molecules, e.g., a non-covalent complex, to an ionized molecule, and to a covalently or non-covalently modified molecule, e.g., modified by phosphorylation, acylation, cross-linking, cyclization, or limited cleavage, which is capable of binding to a target. “Binding composition” may also refer to a molecule in combination with a stabilizer, excipient, salt, buffer, solvent, or additive. “Binding” may be defined as an association of the binding composition with a target where the association results in reduction in the normal Brownian motion of the binding composition, in cases where the binding composition can be dissolved or suspended in solution.

[0019] “Cachexia” is a wasting syndrome involving loss of muscle (muscle wasting) and fat, resulting from a disorder in metabolism. Cachexia occurs in various cancers, chronic pulmonary obstructive disorder (COPD), advanced organ failure, and AIDS. “Cancer

cachexia” is the cachexia that occurs with cancer. Cancer cachexia is characterized by, e.g., marked weight loss, anorexia, asthenia, and anemia. Anorexia is a disorder resulting from lack of motivation to eat, e.g., food aversion (see, e.g., MacDonald, *et al.* (2003) *J. Am. Coll. Surg.* 197:143-161; Rubin (2003) *Proc. Natl. Acad. Sci. USA* 100:5384-5389; Tisdale (2002) *Nature Reviews Cancer* 2:862-871; Argiles, *et al.* (2003) *Drug Discovery Today* 8:838-844; Lelli, *et al.* (2003) *J. Chemother.* 15:220-225; Argiles, *et al.* (2003) *Curr. Opin. Clin. Nutr. Metab. Care* 6:401-406).

[0020] “Conservatively modified variants” applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences or, where the nucleic acid does not encode an amino acid sequence, to essentially identical nucleic acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids may encode any given protein.

[0021] As to amino acid sequences, one of skill will recognize that an individual substitution to a nucleic acid, peptide, polypeptide, or protein sequence which substitutes an amino acid or a small percentage of amino acids in the encoded sequence for a conserved amino acid is a “conservatively modified variant.” Conservative substitution tables providing functionally similar amino acids are well known in the art. An example of a conservative substitution is the exchange of an amino acid in one of the following groups for another amino acid of the same group (U.S. Pat. No. 5,767,063 issued to Lee, *et al.*; Kyte and Doolittle (1982) *J. Mol. Biol.* 157: 105-132):

- (1) Hydrophobic: Norleucine, Ile, Val, Leu, Phe, Cys, or Met;
- (2) Neutral hydrophilic: Cys, Ser, Thr;
- (3) Acidic: Asp, Glu;
- (4) Basic: Asn, Gln, His, Lys, Arg;
- (5) Residues that influence chain orientation: Gly, Pro;
- (6) Aromatic: Trp, Tyr, Phe;
- (7) Small amino acids: Gly, Ala, Ser.

[0022] “Effective amount” encompasses an amount sufficient to ameliorate or prevent a symptom or sign of the medical condition. Effective amount also means an amount sufficient to allow or facilitate diagnosis. An effective amount for a particular patient or veterinary subject may vary depending on factors such as the condition being treated, the



overall health of the patient, the method route and dose of administration and the severity of side effects (see, e.g., U.S. Pat. No. 5,888,530 issued to Netti, *et al.*). An effective amount can be the maximal dose or dosing protocol that avoids significant side effects or toxic effects. The effect will result in an improvement of a diagnostic measure or parameter by at least 5%, usually by at least 10%, more usually at least 20%, most usually at least 30%, preferably at least 40%, more preferably at least 50%, most preferably at least 60%, ideally at least 70%, more ideally at least 80%, and most ideally at least 90%, where 100% is defined as the diagnostic parameter shown by a normal subject (see, e.g., Maynard, *et al.* (1996) *A Handbook of SOPs for Good Clinical Practice*, Interpharm Press, Boca Raton, FL; Dent (2001) *Good Laboratory and Good Clinical Practice*, Urch Publ., London, UK).

[0023] “Exogenous” refers to substances that are produced outside an organism, cell, or human body, depending on the context. “Endogenous” refers to substances that are produced within a cell, organism, or human body, depending on the context.

[0024] “Immune condition” or “immune disorder” encompasses, e.g., pathological inflammation, an inflammatory disorder, and an autoimmune disorder or disease. “Immune condition” also refers to infections, persistent infections, and proliferative conditions, such as cancer, tumors, and angiogenesis, including infections, tumors, and cancers that resist irradiation by the immune system. “Cancerous condition” includes, e.g., cancer, cancer cells, tumors, angiogenesis, and precancerous conditions such as dysplasia.

[0025] “Inflammatory disorder” means a disorder or pathological condition where the pathology results, in whole or in part, from, e.g., a change in number, change in rate of migration, or change in activation, of cells of the immune system. Cells of the immune system include, e.g., T cells, B cells, monocytes or macrophages, antigen presenting cells (APCs), dendritic cells, microglia, NK cells, NKT cells, neutrophils, eosinophils, mast cells, or any other cell specifically associated with the immunology, for example, cytokine-producing endothelial or epithelial cells.

[0026] “Inhibitors” and “antagonists” or “activators” and “agonists” refer to inhibitory or activating molecules, respectively, e.g., for the activation of, e.g., a ligand, receptor, cofactor, gene, cell, tissue, or organ. A modulator of, e.g., a gene, a receptor, a ligand, or a cell, is a molecule that alters an activity of the gene, receptor, ligand, or cell, where activity can be activated, inhibited, or altered in its regulatory properties. The modulator may act alone, or it may use a cofactor, e.g., a protein, metal ion, or small molecule. Inhibitors are

compounds that decrease, block, prevent, delay activation, inactivate, desensitize, or down regulate, e.g., a gene, protein, ligand, receptor, or cell. Activators are compounds that increase, activate, facilitate, enhance activation, sensitize, or up regulate, e.g., a gene, protein, ligand, receptor, or cell. An inhibitor may also be defined as a composition that reduces, blocks, or inactivates a constitutive activity. An “agonist” is a compound that interacts with a target to cause or promote an increase in the activation of the target. An “antagonist” is a compound that opposes the actions of an agonist. An antagonist prevents, reduces, inhibits, or neutralizes the activity of an agonist. An antagonist can also prevent, inhibit, or reduce constitutive activity of a target, e.g., a target receptor, even where there is no identified agonist.

**[0027]** To examine the extent of inhibition, for example, samples or assays comprising a given, e.g., protein, gene, cell, or organism, are treated with a potential activator or inhibitor and are compared to control samples without the inhibitor. Control samples, i.e., not treated with antagonist, are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 25%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

**[0028]** Endpoints in activation or inhibition can be monitored as follows. Activation, inhibition, and response to treatment, e.g., of a cell, physiological fluid, tissue, organ, and animal or human subject, can be monitored by an endpoint. The endpoint may comprise a predetermined quantity or percentage of, e.g., an indicia of inflammation, oncogenicity, or cell degranulation or secretion, such as the release of a cytokine, toxic oxygen, or a protease. The endpoint may comprise, e.g., a predetermined quantity of ion flux or transport; cell migration; cell adhesion; cell proliferation; potential for metastasis; cell differentiation; and change in phenotype, e.g., change in expression of gene relating to inflammation, apoptosis,

transformation, cell cycle, or metastasis (see, e.g., Knight (2000) *Ann. Clin. Lab. Sci.* 30:145-158; Hood and Cheresch (2002) *Nature Rev. Cancer* 2:91-100; Timme, *et al.* (2003) *Curr. Drug Targets* 4:251-261; Robbins and Itzkowitz (2002) *Med. Clin. North Am.* 86:1467-1495; Grady and Markowitz (2002) *Annu. Rev. Genomics Hum. Genet.* 3:101-128; Bauer, *et al.* (2001) *Glia* 36:235-243; Stanimirovic and Satoh (2000) *Brain Pathol.* 10:113-126).

[0029] An endpoint of inhibition is generally 75% of the control or less, preferably 50% of the control or less, more preferably 25% of the control or less, and most preferably 10% of the control or less. Generally, an endpoint of activation is at least 150% the control, preferably at least two times the control, more preferably at least four times the control, and most preferably at least 10 times the control.

[0030] A composition that is "labeled" is detectable, either directly or indirectly, by spectroscopic, photochemical, biochemical, immunochemical, isotopic, or chemical methods. For example, useful labels include  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ ,  $^3\text{H}$ ,  $^{125}\text{I}$ , stable isotopes, fluorescent dyes, electron-dense reagents, substrates, epitope tags, or enzymes, e.g., as used in enzyme-linked immunoassays, or fluorettes (see, e.g., Rozinov and Nolan (1998) *Chem. Biol.* 5:713-728).

[0031] "Ligand" refers, e.g., to a small molecule, peptide, polypeptide, and membrane associated or membrane-bound molecule, or complex thereof, that can act as an agonist or antagonist of a receptor. "Ligand" also encompasses an agent that is not an agonist or antagonist, but that can bind to the receptor without significantly influencing its biological properties, e.g., signaling or adhesion. Moreover, "ligand" includes a membrane-bound ligand that has been changed, e.g., by chemical or recombinant methods, to a soluble version of the membrane-bound ligand. By convention, where a ligand is membrane-bound on a first cell, the receptor usually occurs on a second cell. The second cell may have the same or a different identity as the first cell. A ligand or receptor may be entirely intracellular, that is, it may reside in the cytosol, nucleus, or some other intracellular compartment. The ligand or receptor may change its location, e.g., from an intracellular compartment to the outer face of the plasma membrane. The complex of a ligand and receptor is termed a "ligand receptor complex." Where a ligand and receptor are involved in a signaling pathway, the ligand occurs at an upstream position and the receptor occurs at a downstream position of the signaling pathway.

[0032] "Small molecules" are provided for the treatment of physiology and disorders of tumors and cancers. "Small molecule" is defined as a molecule with a molecular weight

that is less than 10 kD, typically less than 2 kD, and preferably less than 1 kD. Small molecules include, but are not limited to, inorganic molecules, organic molecules, organic molecules containing an inorganic component, molecules comprising a radioactive atom, synthetic molecules, peptide mimetics, and antibody mimetics. As a therapeutic, a small molecule may be more permeable to cells, less susceptible to degradation, and less apt to elicit an immune response than large molecules. Small molecules, such as peptide mimetics of antibodies and cytokines, as well as small molecule toxins are described (see, e.g., Casset, *et al.* (2003) *Biochem. Biophys. Res. Commun.* 307:198-205; Muyldermans (2001) *J. Biotechnol.* 74:277-302; Li (2000) *Nat. Biotechnol.* 18:1251-1256; Apostolopoulos, *et al.* (2002) *Curr. Med. Chem.* 9:411-420; Monfardini, *et al.* (2002) *Curr. Pharm. Des.* 8:2185-2199; Domingues, *et al.* (1999) *Nat. Struct. Biol.* 6:652-656; Sato and Sone (2003) *Biochem. J.* 371:603-608; U.S. Patent No. 6,326,482 issued to Stewart, *et al.*).

[0033] “Specifically” or “selectively” binds, when referring to a ligand/receptor, antibody/antigen, or other binding pair, indicates a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a specified ligand binds to a particular receptor and does not bind in a significant amount to other proteins present in the sample. The antibody, or binding composition derived from the antigen-binding site of an antibody, of the contemplated method binds to its antigen, or a variant or mutein thereof, with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with any other antibody, or binding composition derived thereof. In a preferred embodiment the antibody will have an affinity that is greater than about  $10^9$  liters/mol, as determined, e.g., by Scatchard analysis (Munsen, *et al.* (1980) *Analyt. Biochem.* 107:220-239).

## II. General.

[0034] The present invention provides methods of using polypeptides, nucleic acids, variants, muteins, and mimetics of the IL-23 heterodimer, p19 subunit, p40 subunit, the IL-23 receptor heterodimer, IL-23R subunit, or IL-12Rbeta1 subunit. Also provided are methods for using a hyperkine, i.e., a fusion protein comprising, e.g., the p19 subunit linked to the p40 subunit, as well as nucleic acids encoding the hyperkine (see, e.g., SEQ ID NOs:10 or 11) (Oppmann, *et al.*, *supra*; Fischer, *et al.* (1997) *Nature Biotechnol.* 15:142-145; Rakemann, *et*

*al.* (1999) *J. Biol. Chem.* 274:1257-1266; and Peters, *et al.* (1998) *J. Immunol.* 161:3575-3581).

[0035] Interleukin-23 (IL-23; a.k.a. IL-B30) is a heterodimeric cytokine composed of a novel p19 subunit (SEQ ID NOs: 2 or 4) and the p40 subunit (SEQ ID NOs: 8 or 9) of IL-12 (Oppmann, *et al., supra*). Like p35, p19 requires co-expression of p40 for biological activity (Wiekowski, *et al., supra*). The IL-23 receptor comprises a novel receptor subunit (IL-23R; SEQ ID NO: 6) that binds p19 and IL-12Rbeta1 (SEQ ID NO: 7) that binds p40 (see, e.g., Parham, *et al.* (2002) *J. Immunol.* 168:5699-5708). These two receptor subunits form the functional signaling complex and are expressed on CD4<sup>+</sup>CD45Rb<sup>lo</sup> memory T cells as well as IFNgamma activated bone marrow macrophages (Parham, *et al., supra*).

[0036] Antibodies can be raised to various cytokine proteins, including individual, polymorphic, allelic, strain, or species variants, and fragments thereof, both in their naturally occurring (full-length) forms or in their recombinant forms (see, e.g., SEQ ID NO: 2, 4, 10, or 11). Additionally, antibodies can be raised to receptor proteins (see, e.g., SEQ ID NO: 6) in both their native (or active) forms or in their inactive, e.g., denatured, forms. Anti-idiotypic antibodies may also be used.

[0037] Administration of an IL-23 agonist, i.e., IL-23 or IL-23 hyperkine, can induce, e.g., proliferation of memory T cells, PHA blasts, CD45RO T cells, CD45RO T cells; enhance production of interferon-gamma (IFNgamma) by PHA blasts or CD45RO T cells. In contrast to IL-12, IL-23 preferentially stimulates memory as opposed to naïve T cell populations in both human and mouse. IL-23 activates a number of intracellular cell-signaling molecules, e.g., Jak2, Tyk2, Stat1, Stat2, Stat3, and Stat4. IL-12 activates this same group of molecules, but Stat4 response to IL-23 is relatively weak, while Stat4 response to IL-12 is strong (Oppmann, *et al., supra*; Parham, *et al.* (2002) *J. Immunol.* 168:5699-5708).

[0038] IL-12 and IL-23 engage similar signal transduction mechanisms. IL-23 engaging its receptor complex, activates Jak2, Tyk2, and Stat-1, -3, -4, and -5, as does IL-12. However Stat-4 activation is significantly weaker in response to IL-23 than IL-12. Also, in contrast to IL-12, the most prominent Stat induced by IL-23 is Stat-3 (see, e.g., Parham, *et al., supra*).

[0039] Administration of the p19 subunit of IL-23 can result in, e.g., stunted growth, infertility, and death of animals, as well as inflammatory infiltrates, e.g., in the gastrointestinal tract, lungs, skin, and liver, and epithelial cell hyperplasia, microcytic anemia,

increased neutrophil count, increased serum TNFalpha; and increased expression of acute phase genes in liver; (Wiekowski, *et al.*, *supra*). Enhanced IL-23 expression occurred in immortalized not transformed epithelial cell lines. Thus, IL-23 may provide an early signal of tumor potential *in vivo*.

[0040] Other studies have demonstrated that IL-23 modulates immune response to infection (see, e.g., Pirhonen, *et al.* (2002) *J. Immunol.* 169:5673-5678; Broberg, *et al.* (2002) *J. Interferon Cytokine Res.* 22:641-651; Elkins, *et al.* (2002) *Infection Immunity* 70:1936-1948; Cooper, *et al.* (2002) *J. Immunol.* 168:1322-1327).

[0041] With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual indicates a predisposition for the development of the disease, or can provide a means for detecting the disease prior to the appearance of actual clinical symptoms. Gene expression data is useful tool in the diagnosis and treatment of diseases and pathological conditions (see, e.g., Li and Wong (2001) *Genome Informatics* 12:3-13; Lockhart, *et al.* (1996) *Nature Biotechnol.* 14:1675-1680; Homey, *et al.* (2000) *J. Immunol.* 164:3465-3470; Debets, *et al.* (2000) *J. Immunol.* 165:4950-4956).

### III. Agonists, Antagonists, and Binding Compositions.

[0042] The present invention provides methods of using agonists and antagonist of IL-23. An agonist of IL-23 encompasses, e.g., IL-23, an IL-23 variant, mutein, hyperkine, or peptide mimetic, agonistic antibodies to IL-23R, and nucleic acids encoding these agonists. Antagonists of IL-23 include, e.g., antibodies to IL-23, blocking antibodies to IL-23R, a soluble receptor based on the extracellular region of a subunit of the IL-23R, peptide mimetics thereto, and nucleic acids encoding these antagonists.

[0043] The present invention provides methods of using agonists and antagonists of p19, the complex of p19 and p40, IL-23R, and the complex of IL-23R and IL-12Rbeta1, including binding compositions that specifically bind to proteins and protein complexes of p19, the complex of p19 and p40, IL-23R, and the complex of IL-23R and IL-12Rbeta1.

[0044] An IL-23 hyperkine encompasses, e.g., a fusion protein comprising the polypeptide sequence of p19 and p40, where p19 and p40 occur in one continuous polypeptide chain. The sequences of p19 and p40 may be in either order. The fusion protein may contain a linker sequence, residing in between the sequences of p19 and p40, in one continuous polypeptide chain.

**[0045]** Regions of increased antigenicity can be used for antibody generation. Regions of increased antigenicity of human p19 occur, e.g., at amino acids 16-28; 57-87; 110-114; 136-154; and 182-186 of GenBank AAQ89442 (gi:37183284). Regions of increased antigenicity of human IL-23R occur, e.g., at amino acids 22-33; 57-63; 68-74; 101-112; 117-133; 164-177; 244-264; 294-302; 315-326; 347-354; 444-473; 510-530; and 554-558 of GenBank AAM44229 (gi: 21239252). Analysis was by a Parker plot using Vector NTI® Suite (Informax, Inc, Bethesda, MD). The present invention also provides an IL-23 antagonist that is a soluble receptor, i.e., comprising an extracellular region of IL-23R, e.g., amino acids 1-353 of GenBankAAM44229, or a fragment thereof, where the extracellular region or fragment thereof specifically binds to IL-23. Mouse IL-23R is GenBank NP\_653131 (gi:21362353). Muteins and variants are contemplated, e.g., pegylation or mutagenesis to remove or replace deamidating Asn residues.

**[0046]** Monoclonal, polyclonal, and humanized antibodies can be prepared (see, e.g., Sheperd and Dean (eds.) (2000) *Monoclonal Antibodies*, Oxford Univ. Press, New York, NY; Kontermann and Dubel (eds.) (2001) *Antibody Engineering*, Springer-Verlag, New York; Harlow and Lane (1988) *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 139-243; Carpenter, *et al.* (2000) *J. Immunol.* 165:6205; He, *et al.* (1998) *J. Immunol.* 160:1029; Tang, *et al.* (1999) *J. Biol. Chem.* 274:27371-27378; Baca, *et al.* (1997) *J. Biol. Chem.* 272:10678-10684; Chothia, *et al.* (1989) *Nature* 342:877-883; Foote and Winter (1992) *J. Mol. Biol.* 224:487-499; U.S. Pat. No. 6,329,511 issued to Vasquez, *et al.*).

**[0047]** Purification of antigen is not necessary for the generation of antibodies. Immunization can be performed by DNA vector immunization, see, e.g., Wang, *et al.* (1997) *Virology* 228:278-284. Alternatively, animals can be immunized with cells bearing the antigen of interest. Splenocytes can then be isolated from the immunized animals, and the splenocytes can fused with a myeloma cell line to produce a hybridoma (Meyaard, *et al.* (1997) *Immunity* 7:283-290; Wright, *et al.* (2000) *Immunity* 13:233-242; Preston, *et al.* (1997) *Eur. J. Immunol.* 27:1911-1918). Resultant hybridomas can be screened for production of the desired antibody by functional assays or biological assays, that is, assays not dependent on possession of the purified antigen. Immunization with cells may prove superior for antibody generation than immunization with purified antigen (Kaithamana, *et al.* (1999) *J. Immunol.* 163:5157-5164).

**[0048]** Antibody to antigen and ligand to receptor binding properties can be measured, e.g., by surface plasmon resonance (Karlsson, *et al.* (1991) *J. Immunol. Methods* 145:229-240; Neri, *et al.* (1997) *Nat. Biotechnol.* 15:1271-1275; Jonsson, *et al.* (1991) *Biotechniques* 11:620-627) or by competition ELISA (Friguet, *et al.* (1985) *J. Immunol. Methods* 77:305-319; Hubble (1997) *Immunol. Today* 18:305-306). Antibodies can be used for affinity purification to isolate the antibody's target antigen and associated bound proteins, see, e.g., Wilchek, *et al.* (1984) *Meth. Enzymol.* 104:3-55.

**[0049]** Antibodies will usually bind with at least a  $K_D$  of about  $10^{-3}$  M, more usually at least  $10^{-6}$  M, typically at least  $10^{-7}$  M, more typically at least  $10^{-8}$  M, preferably at least about  $10^{-9}$  M, and more preferably at least  $10^{-10}$  M, and most preferably at least  $10^{-11}$  M (see, e.g., Presta, *et al.* (2001) *Thromb. Haemost.* 85:379-389; Yang, *et al.* (2001) *Crit. Rev. Oncol. Hematol.* 38:17-23; Carnahan, *et al.* (2003) *Clin. Cancer Res.* (Suppl.) 9:3982s-3990s).

**[0050]** Soluble receptors comprising the extracellular domains of IL-23R or IL-12Rbeta1 receptor polypeptides are provided. Soluble receptors can be prepared and used according to standard methods (see, e.g., Jones, *et al.* (2002) *Biochim. Biophys. Acta* 1592:251-263; Prudhomme, *et al.* (2001) *Expert Opinion Biol. Ther.* 1:359-373; Fernandez-Botran (1999) *Crit. Rev. Clin. Lab Sci.* 36:165-224).

#### IV. Therapeutic Compositions, Methods.

**[0051]** The present invention provides IL-23 and anti-IL-23R for use, e.g., in the treatment of proliferative conditions and disorders, including cancer, tumors, angiogenesis, cachexia, cancer cachexia, anorexia, and pre-cancerous disorders, e.g., dysplasia. Nucleic acids are also provided for these therapeutic uses, e.g., nucleic acids encoding IL-23 or IL-23R, or an antigenic fragment thereof, the corresponding anti-sense nucleic acids, and hybridization products thereof. The invention also provides compositions for siRNA interference (see, e.g., Arenz and Schepers (2003) *Naturwissenschaften* 90:345-359; Sazani and Kole (2003) *J. Clin. Invest.* 112:481-486; Pirollo, *et al.* (2003) *Pharmacol. Therapeutics* 99:55-77; Wang, *et al.* (2003) *Antisense Nucl. Acid Drug Devel.* 13:169-189).

**[0052]** To prepare pharmaceutical or sterile compositions including an agonist or antagonist of IL-23, the cytokine analogue or mutein, antibody thereto, or nucleic acid thereof, is admixed with a pharmaceutically acceptable carrier or excipient, see, e.g., *Remington's Pharmaceutical Sciences* and *U.S. Pharmacopeia: National Formulary*, Mack



Publishing Company, Easton, PA (1984). Formulations of therapeutic and diagnostic agents may be prepared by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, e.g., lyophilized powders, slurries, aqueous solutions or suspensions (see, e.g., Hardman, *et al.* (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, McGraw-Hill, New York, NY; Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams, and Wilkins, New York, NY; Avis, *et al.* (eds.) (1993) *Pharmaceutical Dosage Forms: Parenteral Medications*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Tablets*, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) *Pharmaceutical Dosage Forms: Disperse Systems*, Marcel Dekker, NY; Weiner and Kotkoskie (2000) *Excipient Toxicity and Safety*, Marcel Dekker, Inc., New York, NY).

**[0053]** The route of administration is by, e.g., topical or cutaneous application, subcutaneous injection, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intracerebrospinal, intralesional, or pulmonary routes, or by sustained release systems or an implant. Gene transfer vectors, e.g., for the central nervous system, have been described (see, e.g., Cua, *et al.* (2001) *J. Immunol.* 166:602-608; Sidman *et al.* (1983) *Biopolymers* 22:547-556; Langer, *et al.* (1981) *J. Biomed. Mater. Res.* 15:167-277; Langer (1982) *Chem. Tech.* 12:98-105; Epstein, *et al.* (1985) *Proc. Natl. Acad. Sci. USA* 82:3688-3692; Hwang, *et al.* (1980) *Proc. Natl. Acad. Sci. USA* 77:4030-4034; U.S. Pat. Nos. 6,350,466 and 6,316,024).

**[0054]** Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. Preferably, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available (see, e.g., Wawrzynczak (1996) *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.) (1991) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY; Bach (ed.) (1993) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY; Baert, *et al.* (2003) *New Engl. J. Med.* 348:601-608; Milgrom, *et al.* (1999) *New Engl. J. Med.* 341:1966-

1973; Slamon, *et al.* (2001) *New Engl. J. Med.* 344:783-792; Beniaminovitz, *et al.* (2000) *New Engl. J. Med.* 342:613-619; Ghosh, *et al.* (2003) *New Engl. J. Med.* 348:24-32; Lipsky, *et al.* (2000) *New Engl. J. Med.* 343:1594-1602).

**[0055]** Antibodies, antibody fragments, and cytokines can be provided by continuous infusion, or by doses at intervals of, e.g., one day, one week, or 1-7 times per week. Doses may be provided intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, intraspinally, or by inhalation. A preferred dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects. A total weekly dose is generally at least 0.05  $\mu\text{g/kg}$  body weight, more generally at least 0.2  $\mu\text{g/kg}$ , most generally at least 0.5  $\mu\text{g/kg}$ , typically at least 1  $\mu\text{g/kg}$ , more typically at least 10  $\mu\text{g/kg}$ , most typically at least 100  $\mu\text{g/kg}$ , preferably at least 0.2 mg/kg, more preferably at least 1.0 mg/kg, most preferably at least 2.0 mg/kg, optimally at least 10 mg/kg, more optimally at least 25 mg/kg, and most optimally at least 50 mg/kg (see, e.g., Yang, *et al.* (2003) *New Engl. J. Med.* 349:427-434; Herold, *et al.* (2002) *New Engl. J. Med.* 346:1692-1698; Liu, *et al.* (1999) *J. Neurol. Neurosurg. Psych.* 67:451-456; Portielji, *et al.* (20003) *Cancer Immunol. Immunother.* 52:133-144). The desired dose of a small molecule therapeutic, e.g., a peptide mimetic, natural product, or organic chemical, is about the same as for an antibody or polypeptide, on a moles/kg basis.

**[0056]** An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method route and dose of administration and the severity of side affects (see, e.g., Maynard, *et al.* (1996) *A Handbook of SOPs for Good Clinical Practice*, Interpharm Press, Boca Raton, FL; Dent (2001) *Good Laboratory and Good Clinical Practice*, Urch Publ., London, UK).

**[0057]** Typical veterinary, experimental, or research subjects include monkeys, dogs, cats, rats, mice, rabbits, guinea pigs, horses, and humans.

**[0058]** Determination of the appropriate dose is made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, e.g., the inflammation or level of inflammatory cytokines produced.

Preferably, a biologic that will be used is derived from the same species as the animal targeted for treatment, thereby minimizing a humoral response to the reagent.

[0059] Methods for co-administration or treatment with a second therapeutic agent, e.g., a cytokine, steroid, chemotherapeutic agent, antibiotic, or radiation, are well known in the art (see, e.g., Hardman, *et al.* (eds.) (2001) *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10<sup>th</sup> ed., McGraw-Hill, New York, NY; Poole and Peterson (eds.) (2001) *Pharmacotherapeutics for Advanced Practice: A Practical Approach*, Lippincott, Williams & Wilkins, Phila., PA; Chabner and Longo (eds.) (2001) *Cancer Chemotherapy and Biotherapy*, Lippincott, Williams & Wilkins, Phila., PA). An effective amount of therapeutic will decrease the symptoms typically by at least 10%; usually by at least 20%; preferably at least about 30%; more preferably at least 40%, and most preferably by at least 50%.

#### V. Kits and Diagnostic Reagents.

[0060] This invention provides IL-23 proteins, fragments thereof, nucleic acids, and fragments thereof, in a diagnostic kit. Also provided are binding compositions, including antibodies or antibody fragments, for the detection of IL-23 and IL-23 receptor, and metabolites and breakdown products thereof. Typically, the kit will have a compartment containing either a p19 polypeptide, or an antigenic fragment thereof, a binding composition thereto, or a nucleic acid, e.g., a nucleic acid probe or primer. The nucleic acid probe or primer specifically hybridizes under stringent conditions to a nucleic acid encoding p19 or IL-23R.

[0061] The kit can comprise, e.g., a reagent and a compartment, a reagent and instructions for use, or a reagent with a compartment and instructions for use. The reagent can comprise p19, the complex of p19 and p40, IL-23R, the complex of IL-23R and IL-12Rbeta1, or an antigenic fragment thereof, a binding composition, or a nucleic acid. A kit for determining the binding of a test compound, e.g., acquired from a biological sample or from a chemical library, can comprise a control compound, a labeled compound, and a method for separating free labeled compound from bound labeled compound.

[0062] Diagnostic assays can be used with biological matrices such as live cells, cell extracts, cell lysates, fixed cells, cell cultures, bodily fluids, or forensic samples. Conjugated antibodies useful for diagnostic or kit purposes, include antibodies coupled to dyes, isotopes,

enzymes, and metals (see, e.g., Le Doussal, *et al.* (1991) *New Engl. J. Med.* 146:169-175; Gibellini, *et al.* (1998) *J. Immunol.* 160:3891-3898; Hsing and Bishop (1999) *New Engl. J. Med.* 162:2804-2811; Everts, *et al.* (2002) *New Engl. J. Med.* 168:883-889). Various assay formats exist, such as radioimmunoassays (RIA), ELISA, and lab on a chip (U.S. Pat. Nos. 6,176,962 and 6,517,234).

**[0063]** This invention provides polypeptides and nucleic acids of IL-23 and IL-23R, fragments thereof, in a diagnostic kit, e.g., for the diagnosis of proliferative conditions, cancer, tumors, and precancerous disorders, e.g., dysplasia.

**[0064]** Also provided are binding compositions, including antibodies or antibody fragments, for the detection of p19, the complex of p19 and p40, IL-23R, the complex of IL-23R and IL-12Rbeta1, and metabolites and breakdown products thereof. Typically, the kit will have a compartment containing either a IL-23 or IL-23R polypeptide, or an antigenic fragment thereof, a binding composition thereto, or a nucleic acid, such as a nucleic acid probe, primer, or molecular beacon (see, e.g., Rajendran, *et al.* (2003) *Nucleic Acids Res.* 31:5700-5713; Cockerill (2003) *Arch. Pathol. Lab. Med.* 127:1112-1120; Zammattéo, *et al.* (2002) *Biotech. Annu. Rev.* 8:85-101; Klein (2002) *Trends Mol. Med.* 8:257-260).

**[0065]** A method of diagnosis can comprise contacting a sample from a subject, e.g., a test subject, with a binding composition that specifically binds to a polypeptide or nucleic acid of p19, the complex of p19 and p40, IL-23R, and the complex of IL-23R and IL-12Rbeta1. The method can further comprise contacting a sample from a control subject, normal subject, or normal tissue or fluid from the test subject, with the binding composition. Moreover, the method can additionally comprise comparing the specific binding of the composition to the test subject with the specific binding of the composition to the normal subject, control subject, or normal tissue or fluid from the test subject. Expression or activity of a test sample or test subject can be compared with that from a control sample or control subject. A control sample can comprise, e.g., a sample of non-affected or non-inflamed tissue in a patient suffering from an immune disorder. Expression or activity from a control subject or control sample can be provided as a predetermined value, e.g., acquired from a statistically appropriate group of control subjects.

## VI. Uses.

[0066] The present invention provides methods for using agonists and antagonists of IL-23 for the treatment and diagnosis of inflammatory disorders and conditions, e.g., neoplastic diseases, cancers, tumors, angiogenesis, precancerous conditions such as dysplasias, anorexia, cachexia, and cancer cachexia, by modulating immune response.

[0067] The present invention provides methods of treating or diagnosing a proliferative condition or disorder, e.g., cancer of the uterus, cervix, breast, prostate, testes, penis, gastrointestinal tract, e.g., esophagus, oropharynx, stomach, small or large intestines, colon, or rectum, kidney, renal cell, bladder, bone, bone marrow, skin, head or neck, skin, liver, gall bladder, heart, lung, pancreas, salivary gland, adrenal gland, thyroid, brain, ganglia, central nervous system (CNS) and peripheral nervous system (PNS), and immune system, e.g., spleen or thymus. The present invention provides methods of treating, e.g., immunogenic tumors, non-immunogenetic tumors, dormant tumors, virus-induced cancers, e.g., epithelial cell cancers, endothelial cell cancers, squamous cell carcinomas, papillomavirus, adenocarcinomas, lymphomas, carcinomas, melanomas, leukemias, myelomas, sarcomas, teratocarcinomas, chemically-induced cancers, metastasis, and angiogenesis. The invention also contemplates reducing tolerance to a tumor cell or cancer cell antigen, e.g., by modulating activity of a regulatory T cell (Treg) (see, e.g., Ramirez-Montagut, *et al.* (2003) *Oncogene* 22:3180-3187; Sawaya, *et al.* (2003) *New Engl. J. Med.* 349:1501-1509; Farrar, *et al.* (1999) *J. Immunol.* 162:2842-2849; Le, *et al.* (2001) *J. Immunol.* 167:6765-6772; Cannistra and Niloff (1996) *New Engl. J. Med.* 334:1030-1038; Osborne (1998) *New Engl. J. Med.* 339:1609-1618; Lynch and Chapelle (2003) *New Engl. J. Med.* 348:919-932; Enzinger and Mayer (2003) *New Engl. J. Med.* 349:2241-2252; Forastiere, *et al.* (2001) *New Engl. J. Med.* 345:1890-1900; Izbicki, *et al.* (1997) *New Engl. J. Med.* 337:1188-1194; Holland, *et al.* (eds.) (1996) *Cancer Medicine Encyclopedia of Cancer*, 4<sup>th</sup> ed., Academic Press, San Diego, CA).

[0068] The present invention provides methods for treating a proliferative condition, cancer, tumor, or precancerous condition such as a dysplasia, with an agonist or antagonist of IL-23, with at least one additional therapeutic or diagnostic agent. The at least one additional therapeutic or diagnostic agent can be, e.g., a cytokine or cytokine antagonist, such as IL-12, interferon-alpha, or anti-epidermal growth factor receptor, doxorubicin, epirubicin, an anti-folate, e.g., methotrexate or fluoruracil, irinotecan, cyclophosphamide, radiotherapy, hormone

or anti-hormone therapy, e.g., androgen, estrogen, anti-estrogen, flutamide, or diethylstilbestrol, surgery, tamoxifen, ifosfamide, mitolactol, an alkylating agent, e.g., melphalan or cis-platin, etoposide, vinorelbine, vinblastine, vindesine, a glucocorticoid, a histamine receptor antagonist, an angiogenesis inhibitor, radiation, a radiation sensitizer, anthracycline, vinca alkaloid, taxane, e.g., paclitaxel and docetaxel, a cell cycle inhibitor, e.g., a cyclin-dependent kinase inhibitor, a monoclonal antibody, a complex of monoclonal antibody and toxin, a T cell adjuvant, bone marrow transplant, or antigen presenting cells, e.g., dendritic cell therapy. Vaccines can be provided, e.g., as a soluble protein or as a nucleic acid encoding the protein (see, e.g., Le, *et al.*, *supra*; Greco and Zellefsky (eds.) (2000) *Radiotherapy of Prostate Cancer*, Harwood Academic, Amsterdam; Shapiro and Recht (2001) *New Engl. J. Med.* 344:1997-2008; Hortobagyi (1998) *New Engl. J. Med.* 339:974-984; Catalona (1994) *New Engl. J. Med.* 331:996-1004; Naylor and Hadden (2003) *Int. Immunopharmacol.* 3:1205-1215; The Int. Adjuvant Lung Cancer Trial Collaborative Group (2004) *New Engl. J. Med.* 350:351-360; Slamon, *et al.* (2001) *New Engl. J. Med.* 344:783-792; Kudelka, *et al.* (1998) *New Engl. J. Med.* 338:991-992; van Netten, *et al.* (1996) *New Engl. J. Med.* 334:920-921).

[0069] The present invention provides methods for the treatment and diagnosis of anorexia and cachexia, including cancer cachexia. Cachexia is a wasting syndrome that occurs in a number of diseases, including cancer, e.g., cancer of the lung and upper gastrointestinal tract. Cachexia occurs in about half of all cancer patients. Diagnosis of cachexia is by a history of substantial weight loss, loss of appetite, and profound weakness, in the context of advanced disease, and muscle wasting (loss of lean body mass). Cytokines, e.g., IL-6, IL-1, TNFalpha, and IFNgamma, have been associated with cachexia (see, e.g., MacDonald, *et al.*, *supra*; Rubin, *supra*; Tisdale, *supra*; Lelli, *et al.*, *supra*; Argiles, *et al.*, *supra*).

[0070] Also provided are methods of treating extramedullary hematopoiesis (EMH) of cancer. EMH is described (see, e.g., Rao, *et al.* (2003) *Leuk. Lymphoma* 44:715-718; Lane, *et al.* (2002) *J. Cutan. Pathol.* 29:608-612).

[0071] The gastrointestinal tract comprises, e.g., the lips, mouth, esophagus, stomach, small intestines, appendix, large intestines, colon, anus, and rectum. The respiratory tract comprises, e.g., the trachea, bronchioles, bronchi, lungs, alveoli. The reproductive system includes, e.g., the testes, penis, ovaries, uterus, fallopian tubes. The endocrine system

includes, e.g., the pituitary, hypothalamus, pineal gland, thyroid gland, parathyroid, endocrine pancreas, islets, gonads, and adrenal gland.

[0072] The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

[0073] All citations herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0074] Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

## EXAMPLES

## I. General Methods.

[0075] Standard methods in molecular biology are described (Maniatis, *et al.* (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Sambrook and Russell (2001) *Molecular Cloning*, 3<sup>rd</sup> ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Wu (1993) *Recombinant DNA*, Vol. 217, Academic Press, San Diego, CA). Standard methods also appear in Ausbel, *et al.* (2001) *Current Protocols in Molecular Biology*, Vols.1-4, John Wiley and Sons, Inc. New York, NY, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

[0076] Methods for protein purification including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization are described (Coligan, *et al.* (2000) *Current Protocols in Protein Science*, Vol. 1, John Wiley and Sons, Inc., New York). Chemical analysis, chemical modification, post-translational modification, production of fusion proteins, glycosylation of proteins are described (see, e.g., Coligan, *et al.* (2000) *Current Protocols in Protein Science*, Vol. 2, John Wiley and Sons, Inc., New York; Ausubel, *et al.* (2001) *Current Protocols in Molecular Biology*, Vol. 3, John Wiley and Sons, Inc., NY, NY, pp. 16.0.5-16.22.17; Sigma-Aldrich, Co. (2001) *Products for Life Science Research*, St. Louis, MO; pp. 45-89; Amersham Pharmacia Biotech (2001) *BioDirectory*, Piscataway, N.J., pp. 384-391). Production, purification, and fragmentation of polyclonal and monoclonal antibodies is described (Coligan, *et al.* (2001) *Current Protocols in Immunology*, Vol. 1, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) *Using Antibodies*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Harlow and Lane, *supra*). Standard techniques for characterizing ligand/receptor interactions are available (see, e.g., Coligan, *et al.* (2001) *Current Protocols in Immunology*, Vol. 4, John Wiley, Inc., New York).

[0077] Methods for flow cytometry, including fluorescence activated cell sorting (FACS), are available (see, e.g., Owens, *et al.* (1994) *Flow Cytometry Principles for Clinical Laboratory Practice*, John Wiley and Sons, Hoboken, NJ; Givan (2001) *Flow Cytometry*, 2<sup>nd</sup> ed.; Wiley-Liss, Hoboken, NJ; Shapiro (2003) *Practical Flow Cytometry*, John Wiley and Sons, Hoboken, NJ). Fluorescent reagents suitable for modifying nucleic acids, including



nucleic acid primers and probes, polypeptides, and antibodies, for use, e.g., as diagnostic reagents, are available (Molecular Probes (2003) *Catalogue*, Molecular Probes, Inc., Eugene, OR; Sigma-Aldrich (2003) *Catalogue*, St. Louis, MO).

[0078] Standard methods of histology of the immune system are described (see, e.g., Muller-Harmelink (ed.) (1986) *Human Thymus: Histopathology and Pathology*, Springer Verlag, New York, NY; Hiatt, *et al.* (2000) *Color Atlas of Histology*, Lippincott, Williams, and Wilkins, Phila, PA; Louis, *et al.* (2002) *Basic Histology: Text and Atlas*, McGraw-Hill, New York, NY).

[0079] Methods for the treatment and diagnosis of cancer are described (see, e.g., Alison (ed.) (2001) *The Cancer Handbook*, Grove's Dictionaries, Inc., St. Louis, MO; Oldham (ed.) (1998) *Principles of Cancer Biotherapy*, 3<sup>rd</sup> ed., Kluwer Academic Publ., Hingham, MA; Thompson, *et al.* (eds.) (2001) *Textbook of Melanoma*, Martin Dunitz, Ltd., London, UK; Devita, *et al.* (eds.) (2001) *Cancer: Principles and Practice of Oncology*, 6<sup>th</sup> ed., Lippincott, Phila, PA; Holland, *et al.* (eds.) (2000) *Holland-Frei Cancer Medicine*, BC Decker, Phila., PA; Garrett and Sell (eds.) (1995) *Cellular Cancer Markers*, Humana Press, Totowa, NJ; MacKie (1996) *Skin Cancer*, 2<sup>nd</sup> ed., Mosby, St. Louis; Moertel (1994) *New Engl. J. Med.* 330:1136-1142; Engleman (2003) *Semin. Oncol.* 30(3 Suppl. 8):23-29; Mohr, *et al.* (2003) *Onkologie* 26:227-233).

[0080] Software packages and databases for determining, e.g., antigenic fragments, leader sequences, protein folding, functional domains, glycosylation sites, and sequence alignments, are available (see, e.g., GenBank, Vector NTI® Suite (Informax, Inc, Bethesda, MD); GCG Wisconsin Package (Accelrys, Inc., San Diego, CA); DeCypher® (TimeLogic Corp., Crystal Bay, Nevada); Menne, *et al.* (2000) *Bioinformatics* 16: 741-742; Menne, *et al.* (2000) *Bioinformatics Applications Note* 16:741-742; Wren, *et al.* (2002) *Comput. Methods Programs Biomed.* 68:177-181; von Heijne (1983) *Eur. J. Biochem.* 133:17-21; von Heijne (1986) *Nucleic Acids Res.* 14:4683-4690).

## II. Mice and Tumor Induction.

[0081] IL-23 p19 deficient mice were generated as described in Cua, *et al.*, *supra*. Mice specifically lacking in IL-23 (p19KO mice; p19 knockout mice; p19<sup>-/-</sup> mice), p19<sup>+/-</sup> mice, and p19<sup>+/+</sup> wild-type control mice, had a B6/129 F2 background.

[0082] Skin tumors were chemically induced in either wild-type (wt) or IL-23 deficient mice (p19KO mice). Tumors were initiated using 50 micrograms of 7,12-dimethylbenz[a]anthracene (DMBA) followed by a promotion steps consisting of two treatments of 30 micrograms each of TPA per week (see, e.g., Oft, *et al.* (2002) *Nat. Cell. Biol.* 4:487-494).

[0083] With tumor studies with Ep2X1B1-nu/nu mice, tumors metastasize, while cachexia does not occur. The mice die, e.g., from extramedullary hematopoiesis (EMH). With tumor studies with Ep2XB1-Balb/c mice, tumor metastasis does not occur, apparently because of the intact immune system in these mice.

## III. Expression of Subunits of p19 and IL-23R.

[0084] Expression of the p19 subunit of IL-23 and the IL-23R subunit of IL-23 receptor was elevated in a number of cancers, tumors, and cell lines, e.g., cancer of the gastrointestinal tract, reproductive tract, skin, and breast (Table 1).

Table 1. Expression of subunits of p19 and IL-23R by Taqman® analysis, relative to ubiquitin (1.0). The values are from diseased and adjacent normal tissues, where indicated.

Expression of human p19			
normal colon, adjacent	4.8	colon stage I, adenocarcinoma	30.5
normal colon, adjacent	2.0	colon stage II, adenocarcinoma	73.4
normal colon, adjacent	0.8	colon stage II, adenocarcinoma	18.1
normal colon, adjacent	0.21	colon stage III, adenocarcinoma	34.0
normal skin adjacent	2.2	human skin II melanoma	21.8
normal skin adjacent	6.7	human skin II nodular melanoma	16.4
normal skin adjacent	8.4	human skin II nodular melanoma	26.8
normal skin adjacent	9.3	human skin II superficial spreading melanoma	75.1
uterus adjacent	1.6	ovary papillary serous cystadenocarcinoma	55.0
ovary adjacent	1.9	ovary papillary serous cystadenocarcinoma	17.7
breast adjacent	8.2	breast IIB carcinoma, medullary	32.0
breast adjacent	0.6	breast IIA carcinoma, infiltrating duct	3.1
breast adjacent	0.2	breast IIA carcinoma, infiltrating duct	3.9
Expression of human IL-23R			
monocyte/PBMC resting			10.0
leukocytes leukemia SR cell line			415.8
leukocytes leukemia K562 cell line			396.7
leukocytes leukemia MOLT-4 cell line			0.0
leukocytes leukemia HL60 TB cell line			374.1

[0085] RNA from tissues or cell pellets was extracted using RNeasy® columns (Qiagen, Valencia, CA) and treated with Dnase I (Promega, Madison, WI). cDNA were

prepared and used as templates for quantitative real time PCR. cDNA (25 ng) was analysed for expression of a range of genes using GeneAmp® 5700 Sequence Detection System (Applied Biosystems, Foster City, CA). Analysis of cDNA samples from normal and tumor colon and ovary tissue was normalized to expression of the housekeeping gene, ubiquitin.

#### IV. p19 Antagonists Prevent or Reduce Tumors.

[0086] Tumors induced by injected tumor cells or by chemical carcinogenesis, were eradicated or reduced in mice treated antagonists to IL-23, e.g., by treatment with anti-p19 antibody, or by genetic ablation of the p19 subunit (p19KO). p19 is a subunit of IL-23 only, while p40 is a subunit of both IL-23 and IL-12. In contrast, treatment with an IL-12, under some conditions, exacerbated tumors, i.e., resulted in an increase in tumor volume, relative to control mice.

[0087] Tumors in mice resulted in cancer, cancer cachexia, extramedullary hematopoiesis, and death. Treatment of tumor-bearing Balb/c mice with anti-p19 antibody resulted in a halt to increases in tumor volume, while treatment with anti-p40 antibody provoked weight gain of the animal, likely a reversal of cachexia, but an increase in tumor volume (Table 2).

Table 2. Tumor growth in Balb/c mice inoculated with Ep2 (a.k.a. XTb cells) cancer cells (ras-transformed mouse mammary cells).

Antibody treatment	Tumor size (mm <sup>3</sup> )		
	Day 1	Day 11	Day 21
Isotype antibody (8D5)	0 mm <sup>3</sup>	225 mm <sup>3</sup>	500 mm <sup>3</sup>
Anti-p19 antibody (29A2)	0	200	250
Anti-p40 antibody (C17.8)	0	250	1150

[0088] Cancer death and cancer cachexia were induced in mice, where death and weight loss were prevented by anti-p40 antibody. Mice were injected with  $1 \times 10^6$  EpXT tumor cells (s.c.). Tumor bearing nude mice (Ep2XB1 nu/nu) died from lethal lung metastasis, with deaths occurring at from days 22-42 after the injection. Tumor bearing Exp2XB1 Balb/c mice died at about days 22-49 after the injection, where the BalbC/c mice died in absence of lung metastasis. Cachexia was indicated by the decrease in body weight occurring (prior to death). Progressive weight loss occurred, starting at about day 16. The

initial weight, at day 1 was 22-23 grams, while the weight at death was in the range of 16-18 grams.

[0089] Antibody treatment was with C17.8 rat anti-p40 antibody (1 mg/week). With antibody treatment, the Ep2XB1-Balb/C mice (immunocompetant mice), survived until about day 64, after which deaths occurred until day 85. Anti-p40 antibody treatment also resulted in a maintenance of body weight (at about 17 grams) in half of the mice, with a progressive increase in body weight of the remaining mice, to a maximum, within the time frame of the experiment, of 22-23 grams. Thus, anti-p40 antibody resulted in improvement in health, according to survival time and regain of body weight, though anti-p40 could also result in a decline in health, as shown by an increase in tumor size (Table 2).

[0090] Cancer was chemically induced by treatment with DMBA (50 micrograms) and 2 x 30 micrograms tetradecanoylphorbol-13-acetate (TPA) per week (Gschwendt, *et al.*(1991) *Trends Biochem Sci.* 16:167-169). Chemical carcinogenesis treatments were applied to B6/129 wild type mice and to p19KO mice. Wild type mice readily developed tumors but the p19KO mice did not acquire tumors (Table 3).

Table 3. p19KO Mice Resist Chemical Carcinogenesis.

	Initiation with DMBA (50 micrograms); Promotion with TPA (2 x 30 micrograms/week for 13 weeks).		Initiation with DMBA (50 micrograms); Promotion with TPA (2 x 30 micrograms/week for 20 weeks).	
	First tumor occurrence (after TPA)	Tumor number per mouse	First tumor occurrence (after TPA)	Tumor number per mouse
B2/129 wild type mouse	8 weeks	11	8 weeks	8
p19KO mouse	None found in examined time frame.	0	None found in examined time frame.	0

[0091] Separate studies demonstrated that the p19KO prevented tumor formation ,while the p35KO exacerbated tumor formation (Table 4).

Table 4. Influence of p19KO versus p35KO on chemical carcinogenesis.

	Average number of tumors per mouse
C57/129 wild type	10.0
p19KO (C57/129)	0.0
C57B/6 wild type	4.5
p35KO (C57/129)	11.0

[0092] Tissue and cell expression of the subunits of IL-23 and subunits of IL-12 was determined, after carcinogen treatment. DMBA alone, TPA alone, and DMBA with TPA, induced expression of the p19 subunit of IL-23, these chemicals was applied to the mouse's back. For example, two days after treatment with DMBA resulted in an increase in p19 expression from 1.5 (untreated) to 6.3 (at t = 2 days). Expression of p40 increased, but was relatively low in this time interval (0.1 untreated; 0.4 at t = 2 days). Five hours after treatment with TPA resulted in an increase in p19 expression (2.5 control; 15.5 with TPA treatment), but relatively little change in p40 expression (2.0 control; 3.5 with TPA treatment). Five hours after treatment with DMBA plus TPA resulted in large increases in p19 expression (6.0 control; 32.0 DMBA + TPA), but moderate levels of p40 expression (2.0 control; 4.0 DMBA + TPA).

[0093] Response of human keratinocytes to, e.g., DMBA, TPA, and lipopolysaccharide (LPS), was also determined (Table 5). TPA specifically induced p19, with little or no induction of p40, the common subunit of IL-23 and IL-12. LPS induced p19, indicating a role in IL-23 in innate response. Toll-like receptors that bind LPS occur on keratinocytes (see, e.g., Song, *et al.* (2002) *J. Invest. Dermatol.* 119:424-432). Etoposide is an anti-cancer agent that inhibits topoisomerase II and induces apoptosis (see, e.g., Robertson, *et al.* (2000) *J. Biol. Chem.* 275:32438-32443; Karpnich, *et al.* (2000) *J. Biol. Chem.* 277:16547-16552).

Table 5. Response of Human Keratinocytes to Various Additives. N.D. means not detected.

Additive	p19	p40	p35	EBI3 subunit of IL-27 (p28 + EBI3)
Control	1.1	N.D.	0.4	0.01
DMBA	1.0	N.D.	N.D.	N.D.
TPA	1.9	N.D.	0.2	1.25
LPS	4.45	0.05	0.35	0.25
Etoposide	2.5	0.4	1.75	0.6

**[0094]** Anti-p19 antibodies were tested for their effect on the 4T1 mouse breast cancer cell model. Mice were treated with control mIgG1 (27F11) antibody or with anti-p19 antibody (29A2). Tumor growth was monitored on days 1, 3, 4, 5, 6, 7, 8, 9, 10, and 11. Antibodies (1 mg/dose) were administered on days 2, 5, 8, and 10. On day 4, the tumor size of the control antibody treated mouse was about 175 mm<sup>3</sup>, while tumor size of the anti-p19 antibody treated mouse was about 135 mm<sup>3</sup>. Thus, anti-p19 antibody is effective in treating a model of breast cancer. After day 4, tumors in both groups grew at about the same rate, indicating that the antibody dose was not sufficient to counteract the IL-23 expressed by the tumor at later periods in time.

**[0095]** Histology of the Ep2 mouse breast cancer model demonstrated co-localization of IL-23R and NK cells, as determined by staining for p19, which resides bound to IL-23R, and by staining for CD49B, a marker for NK cells. This co-localization occurred in the central part of the tumor, i.e., in the necrotic region. Histology of the Ep2 mouse breast cancer also demonstrated co-localization of p19 and T cells. T cell location was determined by staining for CD3. This co-localization occurred at the peripheral part of the tumor.

#### V. Listing of Sequence Identifiers

- [0096]** SEQ ID NO: 1 is human IL-23p19 nucleic acid sequence.  
**[0100]** SEQ ID NO: 2 is human IL-23p19 amino acid sequence.  
**[0101]** SEQ ID NO: 3 is mouse IL-23p19 nucleic acid sequence.  
**[0102]** SEQ ID NO: 4 is mouse IL-23p19 amino acid sequence.  
**[0103]** SEQ ID NO: 5 is human IL-23 receptor nucleic acid sequence.

[0104] SEQ ID NO: 6 is human IL-23 receptor amino acid sequence.

[0105] SEQ ID NO: 7 is human IL-12Rbeta1 amino acid sequence.

[0106] SEQ ID NO: 8 is human IL-12 p40 amino acid sequence.

[0107] SEQ ID NO: 9 is mouse IL-12 p40 amino acid sequence.

[0108] SEQ ID NO: 10 is mouse IL-23 hyperkine

[0109] SEQ ID NO: 11 is human IL-23 hyperkine.

[0110] All citations herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[0111] Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.